EXHIBIT 20

21

Response Due 6-20-91 ms

☑ This application has been examined ☑ Responsive to communication filed on 9-4-90 and 1-14-91 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter. Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☑ Notice of References Cited by Examiner, PTO-892.
2. ☑ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☑ Claims 1-105 are pending in the application.

   Of the above, claims 1-5, 8-17, 16-25, 28-35, 37-39, 42, 43, 55-74 and 76-105 are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☑ Claims 6, 7, 15, 26, 27, 36, 40, 41, 44-54 and 75 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☑ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____ ; filed on _____.

13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

EXAMINER'S ACTION

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

The incorporation of essential material by reference to a foreign application or foreign patent or to a publication inserted in the specification is improper. Applicant is required to amend the disclosure to include the material incorporated by reference. The amendment must be accompanied by an affidavit or declaration executed by the applicant, or applicant's attorney or agent, stating that the amendatory material consists of the same material incorporated by reference in the referencing application. In re Hawkins, 486 F.2d 569, 179 USPQ 157; In re Hawkins, 486 F.2d 579, 179 USPQ 162; In re Hawkins, 486 F.2d 577, 179 USPQ 167.

Restriction to one of the following inventions is required under 35 U.S.C. § 121:

I. Claims 1-5, 8-14, 16-25, 28-35, 37-39, 42 and 43, drawn to a method of preparing sequences on a solid support, classified in Class 530, subclass 334.

II. Claims 6, 7, 15, 26, 27, 36, 40, 41, 44-54, and 75, drawn to a method of screening sequences on a solid support, classified in Class 436, subclass 518.

III. Claims 55-68 and 76-104, drawn to an apparatus for preparing a plurality of polymers, classified in Class 422, subclass 131.

IV. Claims 69-74, drawn to a substrate for screening for biological activity, classified in Class 436, subclass 518.

V. Claim 105, drawn to a method of synthesizing an RNA or DNA binding sequence for sequencing by hybridization, classified in Class 536, subclass 27.

Inventions of Group I and Groups III and IV are related as process and apparatus for its practice. The inventions are distinct if it can be shown that either: (1) the process as claimed can be practiced by another materially different apparatus or by hand, or (2) the apparatus as claimed can be used to practice another and materially different process. (M.P.E.P. § 806.05(e)). In this case the process as claimed can be practiced by another materially different apparatus, and the apparatuses of Groups III and IV can be used to practice other and materially different processes. The apparatus of Group III does not have the specificity of the process of Group I and could therefore be used to prepare surfaces involving several surfaces of the same polymer, or surfaces involving more than two monomers, and the process of Group I as claimed could involve activators other than energy sources, such as chemical activators. The substrate of Group IV is materially different than the process of Group I because it does not include language specifying selective exposure of parts of its surface to first and second monomers, and further states a specific number of ligands immobilized on its surface. Thus, the apparatus of Group IV is not required to practice the process of Group I, and the

process of Group I is not required to prepare the apparatus of Group IV.

The inventions of Group III and Group IV are related as distinct apparatuses. According to MPEP 803, there are two criteria for restriction between patentably distinct inventions:

1. the inventions must be independent or distinct as claimed; and
2. there must be serious burden on the examiner if restriction is not required.

The apparatuses of Group III and Group IV are independent of one another as:

1. The apparatus of Group III does not require the specific number of immobilized ligands of Group IV; and
2. The apparatus of Group IV does not require the selective activation of the apparatus of Group III.

Inventions of Group II and Groups III and IV are related as process and apparatus for its practice. The inventions are distinct if it can be shown that either: (1) the process as claimed can be practiced by another materially different apparatus or by hand, or (2) the apparatus as claimed can be used to practice another and materially different process. (M.P.E.P. § 806.05(e)). In this case the process as claimed can be practiced by a materially different apparatus which does not involve the specific number of ligands immobilized on its surface

as claimed in Group IV or is prepared by methods differing from those of Groups III and V as illustrated, supra.

Inventions of Group V and Groups III and IV are related as process and apparatus for its practice. The inventions are distinct if it can be shown that either: (1) the process as claimed can be practiced by another materially different apparatus or by hand, or (2) the apparatus as claimed can be used to practice another and materially different process. (M.P.E.P. § 806.05(e)). In this case the process as claimed can be practiced by another materially different process. The apparatuses of Groups III and IV as claimed do not include nucleic acids. The process of Group V also does not include the limitations of the apparatuses of Groups III or IV.

The inventions of Group I, Group II and Group V are related as distinct processes. According to MPEP 803, there are two criteria for restriction between patentably distinct inventions:

1. the inventions must be independent or distinct as claimed; and
2. there must be serious burden on the examiner if restriction is not required.

The processes of Group I, Group II and Group V are independent of one another as:

1. The process of Group I is drawn to a method of preparation, whereas the method of Group II is drawn to a method of

detection. The process of Group I as claimed does not require the screening process of Group II;

2. The process of Group I does not include the nucleic acids of Group V and can be practiced with activators other than photochemical.

Because these inventions are distinct for the reasons given above and have achieved separate status in the art as shown by their different classification and/or their recognized divergent subject matter, restriction for examination purposes as indicated is proper.

During a telephone conversation with Vern Norviel on March 8, 1991, a provisional election was made without traverse to prosecute the invention of Group II, claims 6, 7, 15, 26, 27, 36, 40, 41, 44-54, and 75. Affirmation of this election must be made by applicant in responding to this Office action. Claims 1-5, 8-14, 16-25, 28-35, 37-39, 42, 43, 55-74, and 76-105 are withdrawn from further consideration by the Examiner, 37 C.F.R. § 1.142(b), as being drawn to a non-elected invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition

under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

Claims 6, 7, 15, 26, 27, 36, 40, 41, and 44-54 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Rejections of claims 1, 19, 20, and 21 are included because some of the elected claims in the instant invention depend from them.

Claims 6, 7, 15, 26, 27, 36, 40, and 41 are indefinite because claims 6, 7, and 15 depend from claim 1 and claims 26, 27, 36, 40 and 41 depend from claims 19, 20, or 21 which are drawn to a nonelected invention. It is suggested that the material in the nonelected claims be incorporated into their corresponding dependent claims to clarify them.

Claim 6 is also confusing because it is drawn to a method of screening sequences, yet the claim from which it depends, canceled claim 1, does not clearly describe how polymers are formed on the substrate. Claim 1 provides for the binding of two different monomers to two discrete regions on the substrate, but it does not provide steps for the formation of a sequence on these regions. Process claims should be written as positive method steps which clearly describe what materials are needed to perform the step, what is performed in the step, and what is

achieved by that particular step (see ex parte Erlich 3 USPQd 1011 (1986)).

Claims 15 and 36 are indefinite because it is not clear how the marker is associated with the receptor. Is it covalently bound to the receptor, or does it interact with it in another fashion? Functional claim language describing this association should be used to clarify this claim without introducing new matter as outlined under 35 USC 132.

Claim 19 is confusing because it recites in its preamble "said chemical sequences comprising at least a first and second monomer", yet the body of the claim defines a very different first and second monomer which are bound at different regions on the substrate. The sequence formed at the first region and the sequence formed at the second region both seem to comprise two distinct connected monomers, but the use of the terms first monomer and second monomer in these two different contexts is confusing. Alternate language is suggested (see also the preamble of claim 20; this claim suffers from the same confusion). Claim 19 is also confusing in its recitation of "said first and said second region comprising a substrate protective group" in part a). The insertion of a term such as "each" between "second region" and "comprising" would clarify this claim. In addition, it is not clear that the monomers bind such that the protective group is exposed and not the means by which the monomer binds.

Claim language to this effect would further clarify this claim.

Part a) of claim 20 is confusing. What is meant by "a first and second region deactivating"? Perhaps a comma between "second region" and "deactivating" or alternate language would clarify this phrase. Part B is also confusing as a result of the wording of part a) because it is not clear why the first monomer binds to the second region.

Claim 44 is indefinite in its recitation of "TFA" in part b). Full chemical names should be used instead of abbreviations.

Claim 46 is confusing because it is not clear how the marker is related to the method of detection, or how it is used to detect.

Claim 47 is confusing because it is not clear what purpose the mask serves.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same

person.

Claims 6, 7, 15 and 75 are rejected under 35 U.S.C. § 103 as being unpatentable over Lowe et al.

Lowe et al. disclose devices and methods for preparing said devices in which two or more biochemical species can be selectively bound to sensor surfaces (see column 2, line 54-column 3, line 2, column 3, line 30-column 4, line 7 and column 4, lines 16-32). From column 1, line 50-column 2, line 53, they disclose that antibodies and antigens bound to sensors and their use to screen for the corresponding antigen or antibody are conventional. From column 3, line 30 to column 4, line 7, they disclose that the surface of a sensor is modified by attaching a photoactivatible group to it, activating the group, and covalently bonding a ligand to it. They further disclose that a ligand is a species which is capable of participating in a binding reaction with a suitable partner, such as antigen-antibody or hapten-antibody. In column 4, lines 8-15, they disclose the use of a mask to selectively protect and activate sections of the sensor to immobilize ligand. From column 6, line 57-column 7, line 35, they disclose preferred photoactivatible groups. In column 9, lines 18-60, they disclose preferred conditions for the mask and preferred ligands. In columns 11-13, they disclose assays which involve fluorescent markers.

The instant claims, drawn to a method of screening a

plurality of sequences on a substrate, differ from the prior method of Lowe et al., if at all, in the specific recitation of the steps of the instant method. Lowe et al. do not specifically recite the exact steps of the instant method, nor do they specifically recite marker attached to their receptors. Although Lowe et al. do not recite specific steps, they disclose the immobilization of more than one ligand on a sensor surface (which reads on the first and second monomers of the instant invention) via selective exposure of photoactivatible groups vis a mask. In addition, they teach immobilization of antigens and assay of those antigens by antibody, and the use of fluorescent substrates to detect enzymes. Antibodies labelled with fluorescent substrates are well known to those of ordinary skill in the art for use in immunoassays. Since Lowe et al. teach detection of antigen-antibody pairs with their sensor and teach the use of fluorescent markers with their sensors, one would have expected successful use of labelled antibodies in an immunoassay using the sensor of Lowe et al. No showing of evidence is of record which verifiably establishes any nonobvious or unexpected results. Terms which merely set forth a property inherent for an old reagent are not considered to differentiate the claimed agent from that known to the prior art. In re Pearson 181 USPQ 641.

Claims 26, 27, ~~and~~ 36, 40, and 41 are rejected under 35 U.S.C. § 103 as being unpatentable over Lowe et al. as applied to claims 6, 7,

and 15 above, and further in view of Geysen.

The teachings of Lowe et al. were discussed in detail, supra.

Lowe et al. do not disclose the synthesis of chemical sequences on their sensor prior to performing their assay.

Geysen teaches a method for screening a plurality of catamers (which read on the chemical sequences of the instant invention) to determine the sequence of monomer molecules which corresponds to the epitope which is recognized by an antibody (also called the mimotope). In column 3, lines 6-15, he teaches that the catamers can comprise a number of different types of monomer molecules, including nucleic acids and alpha amino acids. From column 3, line 40 to column 4, line 39, he teaches a method of detecting the mimotope by screening a number of different catamers with an antibody. From column 5, line 43 to column 6, line 55, he teaches methods for synthesizing the catamers. He teaches the activation of a linker attached to a solid support and the binding of a first monomer to the activated linker, followed by activation of the first monomer and binding of a second monomer. He also teaches synthesis of catamers on the solid support which contain different sets of monomers so that a number of different potential mimotopes are present on the solid support. From column 6, line 56-column 7, line 1, he teaches testing of the catamers and further teaches that preferred

methods of detecting antibody binding to the catamers is via RIA or ELISA. In columns 7 and 8, he teaches methods for analysis of antibody binding to particular catamers and synthesis of specific catamers.

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to use the methods of Lowe et al. to selectively synthesis two or more discrete chemical sequences as taught by Geysen on a solid support and screen those sequences using the methods of Geysen and Lowe et al. because both Lowe et al. and Geysen teach methods in which more than one ligand is immobilized on a solid support. Geysen teaches methods for coupling various monomers to appropriate linker molecule to obtain various catamers on a solid support In addition, Lowe et al. discloses methods for selectively activating areas of a substrate and binding of ligand to said activated areas to obtain more than one ligand selectively bound to a solid support. Thus, because Geysen teaches the synthesis a various chemical sequences on a solid support, and Lowe et al. discloses methods for selectively binding ligands to discrete areas of a solid support, one would have expected successful incorporation of the catamer sequence methods of Geysen into the methods of Lowe et al. to obtain a number of chemical sequences which are selectively deposited on a solid support.

Claims 44-54 are rejected under 35 U.S.C. § 103 as being

unpatentable over Lowe et al. in view of Geysen and Patchornik et al.

The teachings of Lowe et al. and Geysen were discussed in detail, supra.

Neither Lowe et al. nor Geysen teach the use nitroveratryloxy carbonyl or nitrobenzyloxy carbonyl as a photoactivatible protecting group in their methods.

Patchornik et al. teaches the use of nitroveratryloxycarbonyl and 2-nitrobenzyloxycarbonyl as photosensitive protecting groups in peptide synthesis.

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to use the photosensitive protecting groups of Patchornik et al. to selectively synthesize peptides on discrete areas of a substrate according to the methods of Geysen and Lowe et al. because Lowe et al. disclose the use of photoactivatible groups to attach ligands to discrete areas of a substrate, and Patchornik et al. teach quantitative yield of peptides and proteins using photoactivatible groups. Because Geysen teaches general methods for synthesizing catamers and Lowe et al. and Patchornik et al. teach the use of photoactivatible groups to conjugate molecules, one would have expected successful use of the photoactivatible groups of Patchornik et al. to synthesize catamers according to the methods of Geysen on the supports of Lowe et al. using the

selective methods of Lowe et al. to synthesize peptides of a particular structure on a discrete area of the support.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jacintha M. Stall whose telephone number is (703) 308-0708.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

JS

Jacintha M. Stall
March 15, 1991

Esther Kepplinger

ESTHER L. KEPPLINGER
SUPERVISORY PATENT EXAMINER
GROUP ART UNIT 182

EXHIBIT 21

United States Patent [19]

Lowe et al.

[11] **Patent Number: 4,562,157**

[45] **Date of Patent: Dec. 31, 1985**

[54] **DIAGNOSTIC DEVICE INCORPORATING A BIOCHEMICAL LIGAND**

[75] Inventors: **Christopher R. Lowe**, Newmarket; **Fergus G. P. Earley**, Solihull, both of England

[73] Assignee: **National Research Development Corporation**, London, England

[21] Appl. No.: **614,121**

[22] Filed: **May 25, 1984**

[30] **Foreign Application Priority Data**

May 25, 1983 [GB] United Kingdom 8314523

[51] **Int. Cl.**[4] **C12M 1/34**; G01N 33/54

[52] **U.S. Cl.** **435/291**; 324/71.1; 435/14; 435/176; 436/526; 436/527; 436/806

[58] **Field of Search** 436/806, 291, 526, 527; 435/176, 291, 14; 324/71.1

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,072,576	2/1978	Arwin	436/806 X
4,238,757	12/1980	Schenck	436/806 X
4,314,821	2/1982	Rice	436/806 X
4,444,878	4/1984	Paulus	436/806 X
4,444,892	4/1984	Malmros	436/806 X

OTHER PUBLICATIONS

Chemical Abstracts, 93:88076h (1980).

Breslow, R. et al., J.A.C.S., 96, 5937–5939 (Sep. 4, 1974).

Primary Examiner—Sidney Marantz

Attorney, Agent, or Firm—Oblon, Fisher, Spivak, McClelland & Maier

[57] **ABSTRACT**

A device useful in diagnostics in which a biochemical species is attached to the surface of a sensor, especially to the gate of a field effect transistor. In such a "BIOCHEMFET" it has been a problem to attach two or more biochemical species to the surface of the sensor, especially to the gates of a multi-gated FET. It has now been found possible to bond a group having a photoactivatable function covalently to the sensor surface, photo-expose the resultant modified surface selectively, e.g. through a mask, and bond the biochemical species, e.g. a hapten, antigen, antibody, lectin or enzyme, to the photoactivated function. In this way the biochemical species becomes attached in selected areas only. In view of the success of this technique realized under conditions of miniaturization, the invention makes possible "printed circuits for proteins".

In addition to the device are the process of manufacture thereto, use for diagnostic purposes and a diagnostic kit of the device and a partner capable of binding to the biochemical species.

18 Claims, No Drawings

DIAGNOSTIC DEVICE INCORPORATING A BIOCHEMICAL LIGAND

This invention relates to a device useful in diagnostics in which a biochemical species is attached to the surface of a sensor. An important embodiment relates to a field effect transistor (FET) having a biochemical species attached to the gate thereof (a BIOCHEMFET).

A field effect transistor is a semiconductive device having two spaced apart regions of the same doping polarity, usually n-type, beteen which a potential difference is applied. These regions are connected by a "gate" of insulative material, usually of silica or silicon nitride. Current flows from one of the regions ("the source") to the other region ("the drain") via an induced conducting channel under the gate and is modulated according to the electric potential applied to the gate. Contact of the gate with a material containing charged species alters the electric potential and therefore the value of the source to drain current, which can be measured. Alternatively the source to drain current can be maintained constant by applying an additional potential to the gate and the additional potential necessary for this purpose can be measured.

The FET has been used mainly as an ion-sensitive device, known as an ISFET. The gate region is overlaid with a membrane capable of interacting selectively with ions present in a solution. That is, the membrane adsorbs ions from the solution which alter the electric potential of the membrane and therefore of the gate.

U.S. Pat. No. 4,020,830 (C. C. Johnson, S. D. Moss and J. A. Janata, assignors to the University of Utah) describes ISFETs for use in measuring the concentration of an enzyme or substrate. A second thin film layer or membrane, having an enzyme or substrate immobilized therein is positioned over the ion-selective membrane. When the membrane containing the enzyme, for example, is contacted with a solution containing the substrate, the substrate diffuses into the membrane and reacts with the enzyme. The reaction is accompanied by a net yield or loss of ions. The ion concentration of the underlying ion-selective membrane then changes, thereby affecting its electric potential and giving rise to a measurable change in an electrical signal.

A short review of ISFETs and their application in clinical chemistry and biology is provided by J. Janata, Analytical Proceedings February 1982, pages 65–68.

In another kind of FET mentioned in U.S. Pat. No. 4,020,830, the gate is covered by a membrane or a hydrophobic polymer, e.g. of polyvinyl chloride or polystyrene, to which an antibody or antigen is covalently bound to the surface of the membrane. The covalent bonding of proteins to membranes is described in UK Patent Specification No. 1,527,772 (The University of Utah), in relation to "immunoelectrodes" in which a sensing electrode is surrounded by a sheath of the membrane. In one example, an electrode was coated with polyvinyl chloride, the polyvinyl chloride swelled with a solvent, dried and reacted first with epichlorohydrin and then with the protein Concanavalin A. The reaction of yeast mannan, a polysaccharide precipitated by Concanavalin A was then monitored. In another example rabbit anti-human 7S gamma-globulin antibody was substituted for Concanavalin A and the binding thereof to human 7S gamma-globulin antigen at pH 5 was monitored.

U.S. Pat. No. 4,238,757 (Schenk, assignor to General Electric Company, New York) describes a FET in which a monomolecular layer of a protein, for example an antibody or antigen, is adsorbed onto the top of the insulating layer of the gate. This is a physically attached Langmuir-Blodgett layer. The reaction of the adsorbed antibody with an antigen to be detected affects the charge concentration in the gate and therefore the drain current.

European Patent Specification No. 75353 (Battelle Memorial Institute) describes a method for the determination of a species (analyte) in solution wherein the analyte is made to react with a specific reactant on a waveguide, thus modifying the optical properties of the waveguide, which are measured and compared with standard reference data obtained from calibrating samples of the analyte. The reactant is a protein such as IgG. To attach it the glass is preferably etched in a grating-like pattern with HF. When the IgG (antibody) is introduced, it bonds to the etched areas. An analyte antigen will then be attracted to the IgG. The discontinuity brought about by the pattern will produce a more ordered pattern of scattered light, thus improving the efficiency with which the scattered light is collected in a photomultiplier tube.

U.S. Pat. No. 4,334,480 (Malmros) describes a semiconductor sensor for assay of an analyte in which a specific binding partner for the analyte is absorbed onto a polyacetylene semiconductor. The polyacetylene repeating units $(=CH—CH—)_n$, where n is a large number, have extensive alternating conjugated pi orbitals which provide the semiconductive effect. In order to eliminate "background" variables, two such polyacetylene devices are preferably electrically balanced in the same Wheatstone bridge circuit.

Japanese Patent Application Publication No. 80.10546 (Asahi Glass K.K.) describes a FET immunosensor in which an antibody or antigen is bonded covalently to the insulated gate. Various methods of bonding are proposed. One method is to react the hydroxyl groups of the silica of the gate with gamma-amino propyltriethoxysilane, whereby the ethoxy groups react with the silica and the amino group is left pendant. The amino group is then reacted with the carboxyl group of an antigen or antibody. Another method proposed is to treat the silica surface with thionyl chloride to convert the hydroxyl groups thereof to chlorine atoms and then to react the antigen or antibody, through a carboxyl or amino group thereof, with the chlorinated silica. A more direct method of bonding mentioned involves the reaction of the hydroxyl groups on the silica with carboxyl or hydroxyl groups of an antigen or antibody, thereby forming ester or ether linkages.

To the applicant's knowledge, the prior art has not proposed any solution to the problem of how to attach two or more biochemical species, such as antigens or antibodies, to pre-defined areas on the surface of a "chip". Such a chip could be fabricated as a multi-gated FET, each gate thereby serving as a sensor, and different biochemical species would be attached to the gates. This multi-gated FET could then be used to detect several different species. It would also be possible to attach some standard or reference biochemical species to each chip so as to provide a "control" for each diagnostic test. Although, the prior art mentions the desirability of making such multicomponent devices, see e.g. U.S. Pat. No. 4,020,830, column 11 line 62 to column 12 line 2 and A. U. Ramsing et al., *Analytica Chimica Acta*

118, 45–52 (1980) at page 51, it is not suggested therein how this can be achieved.

Chemical Abstract 93, 88076 h (1980), referring to Japanese Patent Application Publication No. 80.24603 (Olympus Optical Co. Ltd.), mentions ISFETs having multiple elements on a single substrate.

U.S. Pat. No. 4,242,096 (Oliviera, assignor to 3Ms Company) describes an assay for antigen using a piezoelectric oscillator pre-coated with an antigen. The oscillator is a small quartz wafer having two metal electrodes deposited thereon. When placed in an oscillator circuit the portion of quartz wafer lying between the electrodes vibrates with a precise natural frequency. The oscillator is coated first with antigen and then with a mixture of an appropriate antibody and an analyte antigen. The antigen coating is provided by adsorption by self-crosslinking it on the surface of the oscillator using glutaraldehyde, or by priming the surface with poly(2-hydroxy-3-dimethylamino-1,4-butane). The same wafer can be provided with a plurality of pairs of electrodes, the portion of crystal between each pair having a different characteristic frequency. The oscillator is then coated with a mixture of antigens each serving as a specific binding partner for a different antibody, whereby the same oscillator can be contacted with several different antibodies and multiple assays carried out using the same quartz crystal. No further details are given and it is not clear how one would selectively coat the electrodes with different antibodies.

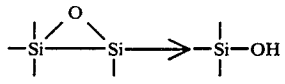
It has now been found that it is possible to position individual biochemical species in selected, i.e. predefined, areas of the surface of sensor such as a chip. According to the present invention, the surface of a sensor is modified by attaching to it, by covalent bonding, a group containing a photoactivatable function, exposing the thus modified surface to photoactivating radiation in selected areas only of the surface, to activate the function, and reacting the function thus activated to bond covalently with a biochemical species. The biochemical species will hereinafter be referred to as ligand, a term which denotes merely that it is capable of participating in some form of binding reaction with a suitable partner. The binding may be of the affinity type, e.g. antigen or hapten-antibody or reversible enzyme-substrate, or it may be a chemical binding.

What is novel and inventive herein comprises the idea and the subsequent practical realisation despite the incredibly small scale involved (even a large test chip typically measures only 3 mm×3 mm). that it is possible to use a photoactivation technique to select minutely small areas, most suitably with the aid of a mask, and thereby successfully attach the biochemical ligand covalently in those areas only.

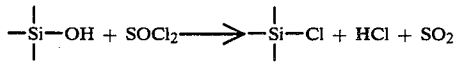
The invention also includes the device so made, which is definable independently of the process of manufacture, as a device which comprises a sensor having a surface to which a group comprising a residue of a biochemical ligand is attached covalently, whereby a physical characteristic of the sensor varies according to whether a binding partner is bound to the ligand, characterized in that the biochemical ligand residue of the group is attached covalently to the surface of the sensor in selected areas only thereof and through a photoactivated covalent linkage.

Also included within the scope of the invention is the use of such a device for assay of a binding partner of the ligand, and a kit useful in diagnostics comprising the device together with at least one binding partner for the biochemical ligand, for testing and/or standardisation of the device.

The invention is particularly applicable to attachment of the group comprising the biochemical ligand residue to a silica surface. A particularly preferred method of attachment is illustrated below, with reference to a silica surface:



(1)



(2)

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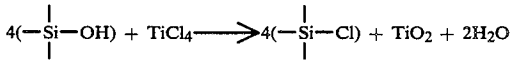

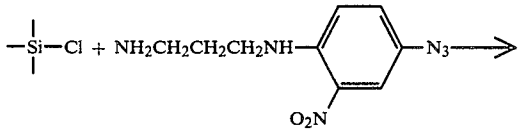

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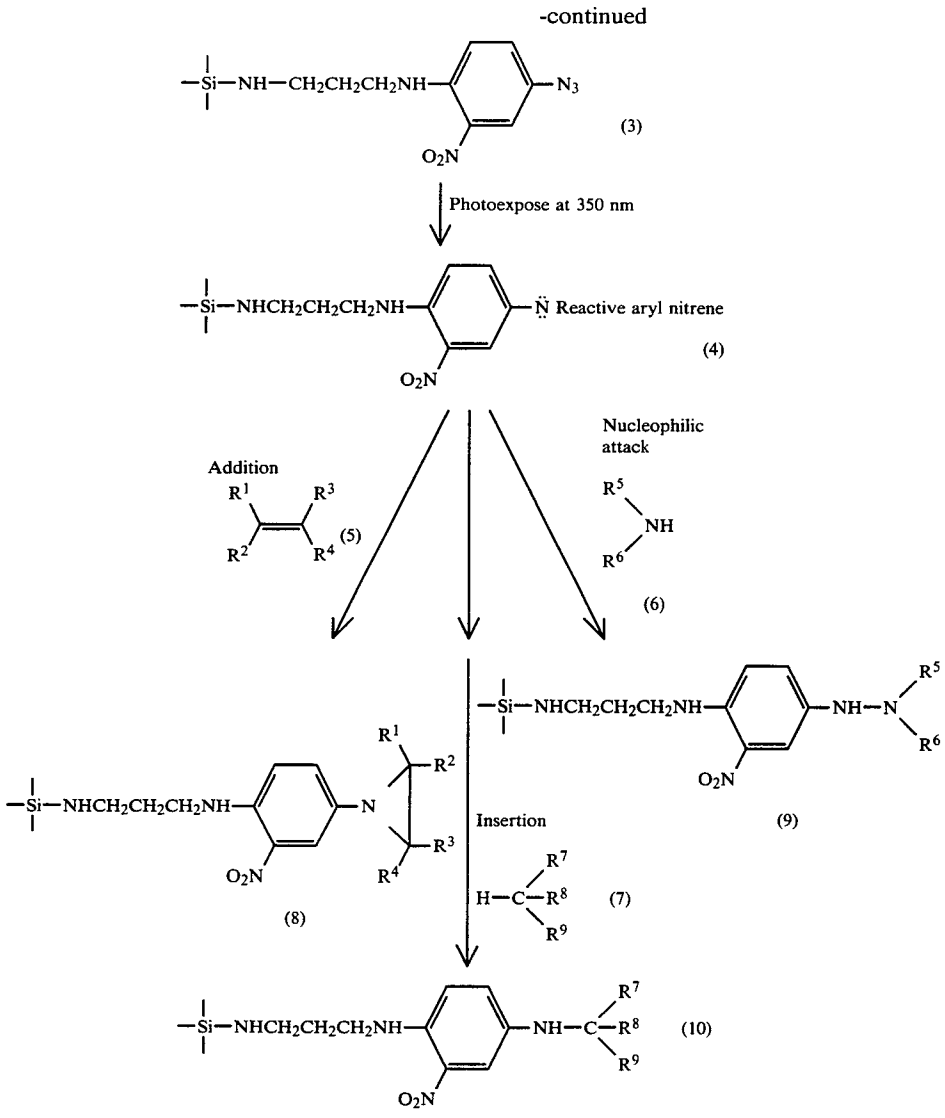
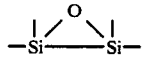


(R^1, R^5 and R^7 represent organic groups; R^2, R^3, R^4, R^6, R^8 and R^9 represent organic groups or hydrogen atoms necessary to complete the biochemical ligand molecules (5), (6) and (7).

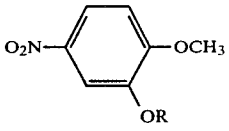
Reference to the illustrated method, the silica produced by thermal deposition in the manufacture of a chip is mainly in an unreactive form, having such linkages as



which must first be hydrolysed to a silanol, —Si—OH, form. This can be done by dipping the surface of the chip for about a minute in 10M solution hydroxide, washing with water and then with a hydrophilic organic solvent such as acetone and drying. Alternatively, several hours refluxing in dilute hydrochloric acid produces a hydrophilic surface, although the effect is not as pronounced in the alkali treatment.

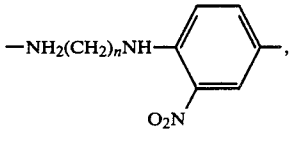
The reactive silica surface is then reacted to replace a hydroxyl group by a more reactive function. In the illustrated method this is a chlorine atom, which can be introduced by reaction with any chlorinating agent capable of nucleophilic displacement at a silicon atom of the hydroxyl group, e.g. thionyl chloride or a titanium chloride.

The chlorinated silica surface is then reacted directly with a compound containing a photoactivatable function at one end or in a branch of the molecule and a function at the other end capable of undergoing a nucleophilic displacement of the chlorine atom on the silicon atom. The latter will ordinarily be an amino group as in the illustrated scheme. The photoactivatable function is preferably provided by an aryl azide, e.g. 3-nitro-4-aminophenyl azide, residue. Other examples of residues providing photoactivatable functions are those derived from ethyl 2-diazomalonyl chloride, nitrophenyl ethers of formula



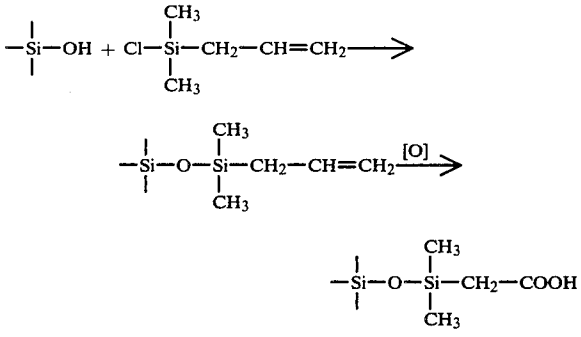
where R is an alkyl group, aromatic ketones as described in *Journal of Biological Chemistry* 249, 3510–3518 (1974), and phosphenyl azides as described by Breslow et al., *Journal of the American Chemical Society*, 96, 5937–9 (1974). Other photoactivatable functions can be provided by the so-called photoaffinity labels, see e.g. Chapter 6, pages 167–179 of the book "Laboratory techniques in biochemistry and molecular biology", Volume 4 Part I: "Chemical Modification of Proteins", by A. N. Glazer, R. J. Delange and D. S. Sigman, general ed. T. S. Work and E. Work, North-Holland Publishing Co. and American Elsevier Publishing Co. Inc. 1975.

A preferred device of the invention has a surface of silica and the biochemical ligand residue is attached to the surface through a group of formula



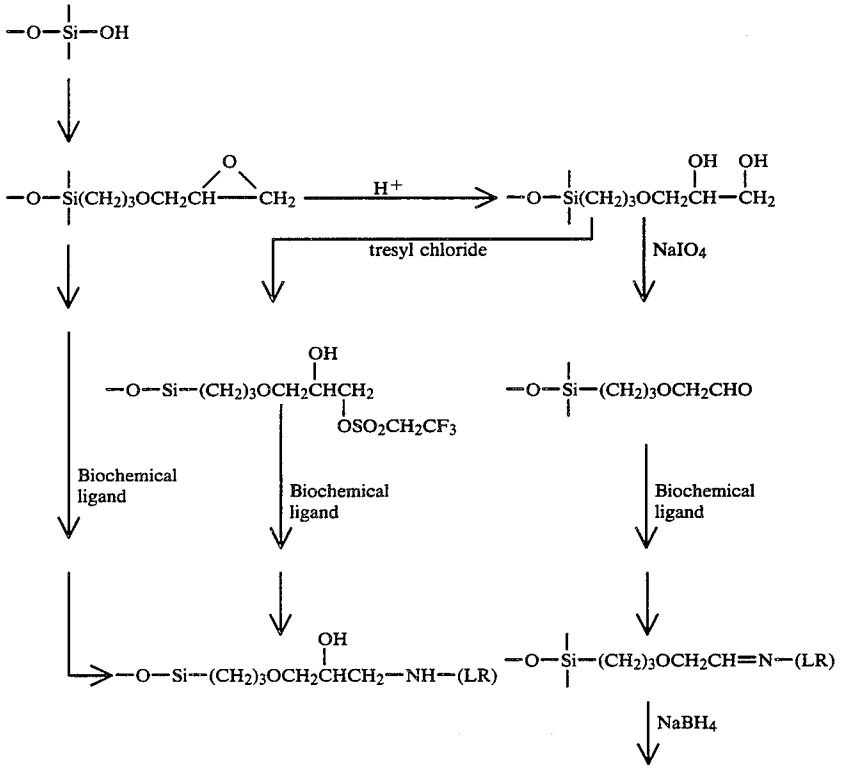
the left-hand end of which is attached to a silicon atom of the surface and the right-hand end of which is attached to the residue of the biochemical ligand, and n is a number from 3 to 12.

Instead of chlorination of the silica and reaction of the chlorinated silica with the group containing the photoactivatable function, it might be desirable to react the silica with a silane, for example allyldimethylchlorosilane, according to the scheme:



The allyl-terminated siloxane is oxidised, e.g. with potassium permanganate dissolved in benzene and a crown ether, to introduce a carboxyl terminus, which is then reacted with a photoactivator having an amino terminus, forming an amide linkage. Other silanes such as gamma-glycidoxypropylmonomethoxydimethylsilane and cyanopropyldimethylchlorosilane could also be exploited to yield bonded phases capable of subsequent chemical modification for attachment of biochemical ligands.

The following reaction scheme illustrates some methods of modifying a silica surface using a gamma-glycidoxypropyl monomethoxysilane, and attaching thereto a group comprising a biochemical ligand (LR=ligand residue):



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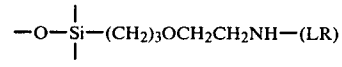


The spacing or bridging arm of the surface-modifying molecule should not be too long, in order to ensure that the sensing function of the device is easily activated by the binding interaction of the biochemical ligand and in order to avoid complications in linking it to the silicon atom. A long carbon-chain spacing arm would create a hydrophobic layer on the silica which might be detrimental, for example, when an enzyme is attached. The enzymic interaction will often be dependent on loss or gain of protons, which will not readily penetrate a hydrophobic layer to reach the sensor surface.

Photoactivation through a mask or screen can be carried out in any manner appropriate for the photoactivating group, i.e. one must choose a radiation, normally in the UV or visible region of the spectrum, to which it is sensitive.

The areas selected by means of the mask for photoactivation can take any appropriate form, depending on how many biochemical ligands are required to be attached. A preferred device is a multi-gated FET having one biochemical ligand attached to each gate and therefore giving an independent signal from other ligands attached to other gates.

For assay purposes a ligand attached to one gate can be left free, i.e. unbound and ready to interact with its partner in the sample to be assayed, while a ligand attached to another gate is blocked from reaction with the binding partner and serves as a control or "reference" gate. In a constant current mode and with a differential amplifier on the same chip, the difference in response of the antigen-sensitive gate and the separate "reference" gate would serve as an internal compensation for any fluctuations in sample composition temperature, pH etc. It is then possible to compare the signals given by the (unbound) ligands, and thereby estimate the amount of binding partner present in the sample.

It is envisaged that the invention can provide a "printed circuit" of a given biochemical ligand, which may have applications outside the diagnostic field, for example, in the development of bio-computers.

The biochemical ligand will normally be a protein and therefore have an amino function. Thus it may be an enzyme, antigen, antibody, receptor protein, other binding protein, or lectin for example. However, it could also be a hapten, co-enzyme, electron mediator or other biologically reactive ligand, particularly one of low molecular weight. Thus, the biochemical ligand could be a sugar or steroid. It may have some other functional group than amino for example a hydroxyl group or carboxyl group in steroid haptens. If the biochemical ligand does not have a group readily reactive with the residue left when the photoactivatable group has been photoactivated, the ligand can normally be further reacted to introduce a more appropriate group.

The silica surface could be replaced by silicon nitride (preferred) or oxynitride or by an oxide of another metal, especially aluminium, titanium (IV) or iron (III) oxides, for example, or any other film, membrane, insulator or semiconductor overlying the device.

The device of the invention need not be based on a FET. Other sensors which can be used include bipolar transistors, semiconductor or other electrodes, piezoelectric crystals, thermoelectric crystals, charge-coupled devices, opto-electronic devices such as integrated optics and waveguide sensors, fibre optic devices, other transducers and magnetic sensors in which a magnetic field strength is measured. Semiconductive devices can contain inorganic semiconductors such as doped silica or organic semiconductors such as polypyrrole. While the physical characteristic sensed is preferably electrical or magnetic, in view of the availability of sensors which amplify changes in electric and magnetic fields, it can in principle be any other physical characteristic, e.g. optical, thermal or the emission or absorption of other radiations.

The following Examples illustrate the invention.

EXAMPLE 1

(1) Synthesis of 4-fluoro-3-nitrophenyl azide

5 grams of 4-fluoro-3-nitroaniline were dissolved in a mixture of 30 ml concentrated HCl and 5 ml water with warming. The solution was filtered and cooled to −20° C. with stirring. (The temperature was maintained between 15° and −20° C. during subsequent production and reaction of the diazonium salt.) An ice-cold solution of 2.4 grams of sodium nitrite in 5 ml water was added dropwise with stirring. The mixture was stirred for a further 30 minutes after completion of the addition, then quickly filtered and the resultant diazonium salt solution was returned to the flask. Stirring was continued during dropwise addition of a solution of 2.2 grams of sodium azide in 8 ml ice-cold water. After completion of this addition, the mixture was allowed to warm and the product was collected by filtration and washed with water. After drying, the product was recrystallised from petroleum ether.

(2) Synthesis of N-(4-azido-2-nitrophenyl)-1,3-diaminopropane

2.5 ml (30 mM) 1,3-diaminopropane was dissolved in 25 ml ethanol. To this solution, was added a solution of 1.82 g (10 mM) 4-fluoro-3-nitrophenyl azide in 25 ml ethanol. The addition was performed dropwise with stirring. The mixture was stirred at room temperature for 16 hours. After this time, 150 ml of water were added and the pH adjusted to between 1 and 2 with concentrated HCl. This solution was filtered to remove the disubstituted amine, cooled on ice and the product was precipitated by the slow addition of concentrated aqueous ammonia with stirring. The product was collected by filtration of the cold mixture, washed with ice-cold water and dried in vacuo.

(3) Pre-treatment of the silica surface

Silicon semiconductor slices having a surface layer of silica were grown thermally and divided into 3 mm square chips. The silica surfaces were hydrolysed by dipping the slices in 10M sodium hydroxide for 1 minute. The slice and solution were not agitated. This treatment was followed by extensive washing in water and slow drying in a stream of nitrogen or argon. This yielded a very hydrophilic surface.

(4) Coupling of N-(4-azido-2-nitrophenyl)-1,3-diaminopropane to the silica surface

Absorbed water on the silica surface was first removed by heating the slices to 105° C. under high vacuum. The slices were then immersed in freshly distilled thionyl chloride. The vessel was sealed and allowed to stand at room temperature for 24 hours. From this stage until completion of coupling of the enzyme, water was rigorously excluded. After 24 hours, excess of thionyl chloride was poured off the slices, the vessel was evacuated and heated to 150° C. under high vacuum to remove adsorbed thionyl chloride, hydrogen chloride and sulphur dioxide from the surface. After cooling, a saturated solution of N-(4-azido-2-nitrophenyl)-1,3-diaminopropane in dry tetrahydrofuran was added and allowed to react in the drak at 40° C. for 24 hours.

(5) Light-dependent coupling of beta-galactosidase to the surface

All operations were performed under red safety light. The slices were removed from the above-mentioned reaction solution and washed in tetrahydrofuran and water and covered with about 1 microliter of a solution of *E. coli* beta-galactosidase, Sigma grade VIII, activity about 1,000 units/ml in 0.1M sodium phosphate buffer of pH 7.5 containing 0.5M NaCl. The enzyme solution was kept on ice before use. The slice was exposed to light from a high pressure mercury vapour lamp (125 watts) through a mask. The incident light was first caused to be reflected from a plastic mirror which absorbs the far ultra-violet light. The mask was of aluminium having a pattern consisting of 5 circular holes arranged in a cruciform layout. The exposure time was 1 minute, after which the slice was washed extensively in ice-cold buffer (0.1M sodium phosphate, pH 7.5 containing 0.5M sodium chloride. The slice was stored in this buffer and could then be exposed to normal lighting.

(6) Assay for beta-galactosidase activity

A saturated solution of 4-methylumbelliferyl beta-D-galactopyranoside was prepared in the above buffer and spread on the surface of the exposed slice. The slice was placed in a dark box under a short-wave ultra-violet lamp. Care was taken not to agitate the slice. After several minutes, blue florescence was observed, the intensity of which reproduced the pattern of 5 holes of the mask. The fluorescence is due to the release of 4-methylumbelliferone from the hydrolysis of 4-methylumbelliferyl-beta-D-galactopyranoside by beta-galactosidase.

EXAMPLE 2

Example 1 was repeated, using an alternative procedure for the chlorination of the silica surface. After pre-treatment of the slices and drying as described previously, the slices were placed in 10 ml dry toluene, to which was added 0.5 ml titanium (IV) chloride. The reaction was allowed to proceed overnight. The slices were then removed, washed with dry toluene and placed in a tetrahydrofuran solution of N-(4-azido-2-nitrophenyl)-1,3-diaminopropane as described previously. Again, great care was taken to exclude moisture.

EXAMPLE 3

(1) Pre-treatment of the silica surface

The precedure of Example 1, stage (3) was repeated, using a chip coated with silicon nitride instead of silica.

(2) Reaction of allyldimethylchlorosilane with the surface of the chip

The slices were heated to 150° C. under high vacuum to remove absorbed water on their surfaces. They were then immersed in 10 ml of sodium-dried toluene containing 1 ml of allyldimethylchlorosilane and 0.1 ml dry pyridine. The reaction vessel was filtered with a condenser and drying tube to exclude moisture and the toluene is refluxed for approximately 3 hours. The slices were then washed exhaustively in benzene, followed by toluene and finally acetone. The hydrophobicity of the resultant surfaces indicated that the reaction had been successful.

(3) Oxidation of the allyl group

10 mg of the macrocyclic polyether known as "18-crown-6" was dissolved in 100 ml benzene. The solution was shaken vigorously with a concentrated aqueous solution of potassium permanganate until the purple colour of the benzene reacted a maximum intensity. The benzene layer was separated and filtered through a 2 micrometer "Millipore" (Registered Trade Mark) filter and applied to the surface of the slices. After 2 hours at room temperature the slices were washed exhaustively in benzene, followed by toluene and finally acetone. The surface reverted to a hydrophilic character, indicating the presence of the desired carboxylic acid group in place of the terminal vinyl part of the allyl group.

(4) Coupling of the N-(4-azido-2-nitrophenyl)-1,3-diaminopropane to the surface

The slices were washed in dry tetrahydrofuran and subsequently incubated with 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (0.5 mg/ml in dry tetrahydrofuran) for 1 hour at 25° C. The slices were washed exhaustively with dry tetrahydrofuran and incubated with a saturated solution of N-(4-azido-2-nitrophenyl)-1,3-diaminopropane in dry tetrahydrofuran in the dark at 40° C. for 24 hours.

(5) Light-dependent coupling of beta-galactosidase to the surface

(6) Assay for beta-galactosidase activity

These steps were carried out as in Example 1.

EXAMPLE 4

Modified and unmodified silicon chips (4 mm×4 mm) prepared as described above were placed in PTFE housing and held firmly by rubber O-rings. Electrical contact to the rear face of the chip was achieved by grinding a small amount of gallium/indium eutectic in with a diamond pen in order to remove the SiO_2 layer grown during storage and contacting with a brass/silver rod to which electrical contact was made in the normal way.

An Ag/AgCl microreference electrode for use with silicon chips was fabricated by pulling out 3 mm soda glass tubing to a fine capillary 0.2 mm diameter. The capillary was sealed at the fine end with a plug of 2% (w/v) agarose saturated with KCl to act as a salt bridge

and sealed at the other with an Ag/AgCl wire in saturated AgCl/KCl. The potential of the microreference electrode was tested versus SCE at various pH values using a high input impedance (10^4 ohms) Keithley Model 642 electrometer. It was found that the drift in the Ag/AgCl microreference electrode was less than 1 mV per decade in pH and was therefore considered acceptable to act as a reference electrode for the modified chips.

Sodium acetate/sodium tetraborate buffers of equiionic strength but different pH values were used at 20.5° C. in order to assess the pH response of SiO_2 and Si_3N_4 coated chips both before and after surface silanization and derivatization as described above. The pH response of the electrode at constant ionic strength was substantially linear, giving a slope of approximately 35 mV per pH unit over the pH range 3.5 to 7 for Si_3N_4-coated chips and approximately 16 mV per pH unit over the pH range 2 to 9 for SiO_2-coated chips. The linearity was unaltered on subsequent silanization. This observation opens the way to covalently bonding such enzymes as penicillinase to silanized oxide, oxynitride or nitride with retention of the pH response and thus of fabricating an enzyme-coated FET.

EXAMPLE 5

Example 3, steps (1) to (6) were repeated except that alkaline phosphatase was used in place of beta-galactosidase and 4-methyl umbelliferyl phosphate was used as the substrate generating fluorescence (by hydrolysis of the phosphate by alkaline phosphatase).

We claim:

1. A device for diagnostics comprising:

a sensor having a surface to which a biochemical ligand is attached covalently, in a manner effective to cause a physical characteristic of the sensor to vary according to whether a binding partner becomes bound to the ligand, wherein the biochemical ligand is attached covalently to the surface of the sensor in selected areas only thereof and through a photoactivated covalent linkage.

2. A device according to claim **1**, wherein the biochemical ligand is an antigen or hapten and a physical characteristic of the sensor varies according to whether or not an antibody becomes bound to the antigen or hapten.

3. A device according to claim **1**, wherein the biochemical ligand is an antibody and a physical characteristic of the sensor varies according to whether or not an antigen or hapten becomes bound to the antibody.

4. A device according to claim **1**, wherein the biochemical ligand is an enzyme and a physical characteristic of the sensor varies according to whether or not a substrate becomes bound to the enzyme.

5. A device according to claim **1**, wherein the biochemical ligand is a co-enzyme, steroid, sugar, electron mediator or other low molecular weight biochemical.

6. A device according to claim **1**, wherein the surface of the sensor is of an inorganic oxide, oxynitride or nitride.

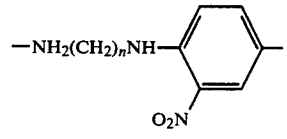
7. A device according to claim **6**, wherein the surface is of silica or silicon nitride.

8. A device according to claim **6**, wherein the surface is of iron (III) oxide and the sensor measures magnetic field strength.

9. A device according to claim **6**, wherein the surface is of silica or silicon nitride and the sensor is a field effect transistor.

10. A device according to claim **1**, wherein the photoactivated covalent linkage is provided by the photolysis of an aryl azide group.

11. A device according to claim **10**, wherein the surface is of silica and the biochemical ligand is attached to the surface through a group of formula:



the left bond is attached to a silicon atom of the surface and the right bond is attached to the biochemical ligand, and n is an integer from 3 to 12.

12. A device according to claim **1**, wherein the sensor is a field effect transistor, the surface is of silicon nitride and the photoactivated covalent linkage is an aryl azide group.

13. A process of preparing the device claimed in claim **1**, which process comprises modifying the surface of the sensor by attaching to it by covalent bonding a group containing a photoactivatable function, exposing the thus modified surface to photoactivating radiation in selected areas only of the surface to activate the photoactivatable function and reacting the photoactivatable function thus activated to bond covalently the biochemical ligand.

14. A process according to claim **13**, wherein the modified surface is exposed to the photoactivating radiation through a mask.

15. A process according to claim **13**, wherein different biochemical ligands are attached to different areas of the same surface.

16. A process according to claim **13**, wherein the sensor is a field effect transistor, the surface is of silicon nitride and the photoactivatable function is an aryl azide group.

17. A kit for diagnostics comprising the device claimed in claim **1** and at least one binding partner for the biochemical ligand for testing or standardisation of the device.

18. The device of claim **1** wherein said physical characteristic is an electrical, magnetic, optical or thermal characteristic.

* * * * *